Prevalence of hereditary hyperferritinemia-cataract syndrome in blood donors and patients with cataract

We screened 3,249 blood donors and 12,916 patients with cataract to get insights into the frequency of hereditary hyperferritinemia cataract syndrome (HHCS) in subjects with unexplained hyperferritinemia and/or cataract. No mutation in the iron responsive element of the L-ferritin gene was found in subjects who met the established inclusion criteria. HHCS appears to be a relatively rare condition, even in selected patients.

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HHCS is an autosomal dominant disease, first described in 1995 in Italy and France,[1,2] and later in several continents. HHCS is due to heterogeneous mutations in the iron responsive element (IRE) of L-ferritin gene, a *cis*-acting non-coding mRNA stem-loop structure which interacts with protein sensors of cellular iron status, the iron regulatory proteins (IRPs).[3] Mutations in the L-ferritin IRE disrupt the negative control of L-ferritin synthesis through the interaction between IRE and IRPs, resulting into constitutive up-regulation of ferritin synthesis in serum and tissues, including the lens.[4] Mutations in the upper hexanucleotide (CAGUGX) loop sequence of the IRE generally determine ferritin levels >700 μg/L and early development (i.e. within the fourth decade) of bilateral cataract.[5] However, mutations in the lower stem have been described to cause slight hyperferritinemia (range 350-650 μg/L) and asymptomatic lens opacities.[6]

While it is now accepted that hematologists and ophthalmologists should consider HHCS in subjects with hyperferritinemia and/or early-onset cataract, the prevalence of HHCS in these clinical conditions is unknown. Our aims were to evaluate: 1) the prevalence of HHCS in subjects from the general population with unexplained hyperferritinemia; 2) the prevalence of HHCS in subjects with juvenile cataract; and 3) the possible contribution of mutations in the L-ferritin IRE to the pathogenesis of age-related cataract, which involves multiple gene-gene/gene-environmental interactions.[7] We used different approaches, summarized as follows:

1) *General population*: we examined 3,246 blood donors (2,159 males, 1,087 females; age range 18-65 years) referring to the Hospital of Bussolengo (Verona, Northern Italy) during a one-year period. Serum ferritin was measured in all of them. Forty-seven had ferritin above the established cut-off (300 μg/L; range: 304-679). They were recalled by phone and 43 agreed to have a slit-lamp examination. Thirteen of them had some spotty opacities in the lens. Their DNA was examined for IRE mutations by a recently validated double-gradient denaturing gradient gel electrophoresis (DG-DGGE) assay.[8]

2) *Juvenile cataract*: we reviewed the registries of all interventions for cataract performed at the Ophthalmology Department of the University of Parma (Northern Italy) during the period 1995-2000. From 11,685 patients we selected 31 patients younger than 41 years at diagnosis. Sixteen subjects with obvious causes of cataract (i.e. diabetes, trauma, corticosteroid therapy) were further excluded. The remaining 15 were examined for IRE mutations by DG-DGGE.

3) *Age-related cataract*: we added ferritin to the routine laboratory evaluation of all consecutive patients (n=1,231; male 489, females 742; mean age: 73.4 years) scheduled to undergo cataract surgery during a one-year period at the Ophthalmology Department of the University of Verona. Forty of them had ferritin levels ≥ 300 μg/L. Twelve were excluded because of the presence of obvious conditions associated with hyperferritinemia (i.e. cancer, hepatic or inflammatory disease). The remaining 26 (21 males, 5 females, mean age 70.7 years, ferritin range: 419-1060 μg/L) were examined for IRE mutations by DG-DGGE.

The results are shown in Table 1. No mutation in the L-ferritin IRE was found in the fifty-four subjects who met the established inclusion criteria.

Recently, mutations in genes encoding for several proteins of the lens (i.e. crystallins) have been associated with autosomal dominant cataract.[9] Rosochova *et al.*[10] previously did not find biochemical features of HHCS in 19 patients with bilateral cataract operated before the age of 51 years old, selected from about 3,000 cases in Geneva (Switzerland). Accordingly, our data from Parma suggest that HHCS is not a frequent cause of juvenile cataract. Similarly, since we found no case of HHCS in patients with age-related cataract, our results do not support the hypothesis that mutations in the L-ferritin IRE contribute to the pathophysiology of this common condition.

No data exist about the prevalence of HHCS in the general population. Caution is needed in considering blood donors as representative of the general population, especially in studies on iron metabolism, which is influenced by regular donations. However, this limitation does not apply to our study, since ferritin levels in HHCS are high regardless of body iron status.[1] As no case of HHCS was found in more than 3,200 subjects, a clear-cut calculation of the prevalence of HHCS in the general population remains difficult, and would require a larger sample size. Another reason for caution lies in the fact that we tested only subjects

Table 1. Characteristics of the three populations studied.

Baseline population	*Inclusion criteria*	*Exclusion criteria*	*Subjects studied for L-ferritin IRE-mutations*	*IRE-mutations*
Blood donors referring during a 1-year period (n= 3,246)	Serum ferritin levels ≥ 300 μg/L and even minimal opacities in the lens	–	13	None
Surgical registry for cataract covering a 6-year period (n= 11,685)	Juvenile cataract (age ≤ 40 years)	Any obvious cause (diabetes mellitus, trauma, corticosteroid therapy)	15	None
Consecutive patients referred for age-related cataract surgery during a 1-year period (n= 1,231)	Serum ferritin ≥ 300 μg/L	Hepatic or systemic inflammatory diseases,cancer	26	None

with clinical signs, possibly underestimating the presence of IRE mutations with low penetrance. Nevertheless, our data on 16,162 subjects suggest that HHCS is a relatively rare condition, even in the setting of selected ophthalmological and/or hyperferritinemic patients.

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Risk factors for hyperbilirubinemia and gallstones in Chinese patients with β thalassemia syndrome

We studied the relationship between jaundice and gallstones and Gilbert alleles (Gly71Arg: 27.8% and $(TA)_7$: 19.6%) in 94 Chinese patients with thalassemia major (TM) and 33 with thalassemia intermedia (TI). Determinants of bilirubin level included age, transfusion (TI>TM) and genetic profile ($\alpha\alpha\alpha/\beta^0$ > β^+/β^0 > β^0/β^0 in TI, β^+/β^0 > β^0/β^0 in adult TM, Gilbert homozygotes > others in TM and TI, Gilbert heterozygotes > wild type in TM). Determinants of gallstones (39%) included age, TI and Gilbert alleles. We conclude that the finding of unusually high bilirubin may indicate either homozygous Gilbert genotype or hemolytic thalassemia genotypes.

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Gilbert's syndrome is caused by recessive alleles for uridine diphosphate-glucuronosyl transferase 1 (UGT1*1) enzyme. It is a reported cause of jaundice and gallstones in thalassemia patients.[1,2] Published studies, however, involved patients with relatively homogeneous Gilbert alleles e.g. $(TA)_7$ and β thalassemia genes e.g. 39 C/T and HbE mutations. The situation in the Southern Chinese population is complicated by heterogeneous β gene defects, α thalassemia co-inheritance, and multiple Gilbert alleles, mainly $(TA)_7$ and Gly71Arg substitution.[3] We revisited the Gilbert/thalassemia interaction in 127 Chinese with clinically defined thalassemia major (TM, n=94) and thalassemia intermedia (TI, n=33) syndromes.[4] Mean unconjugated bilirubin levels were measured. Molecular methods were used to define the thalassemia gene defect,[4, 5] and to detect the two common Chinese Gilbert alleles (Figure 1).[3]

The TI cases were heterogeneous (9 HbE/β^0, 7 $\beta^0/\alpha\alpha\alpha$, 3 β^0+unknown defect, 4β^+/β^+,10 β^+/β^0), while the TM cases included 63 β^+/β^+ and 31 β^+/β^0 cases.[4] The $(TA)_7$ and Gly71Arg alleles were found in 25 (19.6%, 2 homozygous) and 34 cases (26.8%, 6 homozygous), respectively, with 3 double heterozygotes. The median age of patients with TI was higher than that of the patients with TM (32.6 vs 20.0 years; p<0.001), but there was no difference in the incidence of Gilbert alleles (p=0.684). For all patients, serum bilirubin levels increased with age (p<0.001). The median bilirubin level was higher among TI cases than among TM ones (p<0.001). Patients carrying Gilbert alleles had higher bilirubin levels (p=0.027); this effect was mainly contributed by homozygotes and double heterozygotes (p=0.002). The heterozygote effect was evident only in less jaundiced TM cases (p=0.022), but not in TI cases (p=0.68). Apart from Gilbert alleles, the thalassemia genotype was also significant. Higher bilirubin levels were found in β^+/β^0 adult TM cases (p=0.047) and $\beta^0/\alpha\alpha\alpha$ TI cases (p=0.021). Neither the transfusion frequency nor baseline hemoglobin significantly affected bilirubin levels. Gallstones were found by ultrasound or magnetic resonance imaging in 26 of 78 studied patients (median age 23, range 5-66 years), while 8 patients had already surgical stone removal. The prevalence of gallstones increased with age (p<0.001), TM (p=0.002), bilirubin level (p<0.001) and Gilbert genotype (p=0.003). On multivariate analysis, significant predictive factors of bilirubin level included age (p=0.001), Gilbert genotype (p=0.003) and TI (p<0.001). However, only age (p<0.001) and Gilbert genotype (p=0.002) were important gallstone determinants, and the importance of bilirubin level was lost (p=0.052).

Both bilirubin production (hemolysis) and clearance (glucuronidation) are genetically determined in thalassemia patients.[1,2,6] Defective glucuronidation in carriers of Gilbert alleles is known to aggravate jaundice in all hemolytic anemias, including thalassaemia, G6PD deficiency[7] and spherocytosis.[8] The genotype-phenotype relationship in thalassemia is